Dkt. 00156

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Group Art Unit: 1712

NICOLE BRU-MAGNIEZ et al Examiner: R. Sellers

Serial No.: 09/600,895

Filed: September 19, 2000

For: NOVEL SURFACTANT COPOLYMERS
BASED ON METHYLIDENE MALONATE

DECLARATION OF GERARD RIESS UNDER 37 CFR § 1.132

Honorable Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr H. Gerard RIESS, do hereby declare as follows:

I am a named inventor of the above-identified patent application.

I am Emeritus Professor at the University of Upper Alsace (France).

I received a Ph.D. in chemical engineering from the University of Strasbourg in 1957, and have been a professor at the University of Upper Alsace since 1959. In addition, I have published 245 articles, of which about 200 appeared in internationally distributed journals, have participated in 38 patents, given papers at 545 conferences and seminars, of which about 400 were given outside France at international

meetings, and supervised 92 Ph.D. theses in chemical engineering.

The present patent application describes the formation of copolymers from two separate types of monomer. A copolymer contains at least two different monomers covalently bound to one another in a macromolecule. In the case of a block- or graft copolymer, a polymer chain AAAA..., derived from monomer A, is covalently bound to a polymer chain BBBB..., derived from monomer B, to give a molecule of structure ...AAAABBBB... or ..AA(BBBB)AA..., wherein the polymer chains are derived from A monomers or from B monomers, but not from both mixed together (ABABAB).

The cited document WO 96/25954 (U.S. Patent 6,106,807 of Albayrak et al) describes the polymerization of a methylenemalondiester in an aqueous gas-saturated buffer solution (pH between 5 and 8). Among various additives which can be added to the reaction medium in Albayrak et al are surface-active substances. However, the function of such surface-active substances here is to promote the separation of polymer microparticles after their formation, i.e. after the polymerization reaction has occurred. One of the surface-active substances which may be used in this context is Pluronic F 68, which is a triblock copolymer of the following structure:

$$HO-(CH_2-CH_2-O)_n-(CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-O)_m-(CH_2-CH_2-O)_n-H$$

with n = 76 and m = 30.

The Office Action of August 26, 2003 considers the claimed invention to be unpatentable over Albayrak et al under 35 U.S.C. § 103, this rejection being based on the possibility

that copolymers containing both poly(methylidenemalonate) (hereinafter "PMM") and Pluronic could be formed under the conditions taught by Albayrak et al.

A genuine and serious research effort was made by a research team that I lead to identify any copolymer species which might be produced when PMM is formed by polymerisation of the corresponding monomer in the presence of Pluronic® F68.

The chromatographic techniques most often used to separate polymer species in such cases were found to be difficult to apply to solve this particular problem, and we therefore used two alternative methods (MALDI-TOF mass spectrometry, and solvent precipitation followed by NMR).

The research team applied the methods described by Albayrak et al for the polymerization of 1-ethoxycarbonyl, 1-ethoxycarbonylmethylene-oxycarbonylethane monomer (the monomer giving rise to PMM), in the presence of Pluronic® F68, and followed the process described in example 15 of the patent. The experimental conditions, which reproduce the polymer preparation protocol given at column 4, lines 54-63 in Example 7 (without the final separation steps of lines 63-67) are set out in Appendix A, under the heading "Batch A" in the section entitled "Materials and Methods".

Initially, we attempted to study by classical chromatographic methods (Gel Permeation Chromatography (GPC) and High-Performance Liquid Chromatography (HPLC)), whether or not peaks which could be attributed to copolymers could be formed in the Albyrak et al method. However, it was very difficult by these methods to clearly distinguish PMM peaks from Pluronic peaks (the two overlapped), and these methods,

although in principle useful for studying this kind of problem, were not helpful in studying this particular problem.

Therefore, we used two other methods, namely mass spectrometry (in its MALDI-TOF variant) and nuclear magnetic resonance (NMR), in an attempt to identify (and in the case of NMR, quantify) any copolymer formed.

In each case, we compared the results obtained by the polymerisation to form PMM in the presence of Pluronic (batch A), with the results obtained when PMM is formed by polymerisation in the absence of Pluronic (batch B-control).

In the case of analysis by MALDI-TOF, three different mass spectra were recorded (see Appendix A-1), namely (i) PMM formed by polymerisation in the presence of Pluronic (batch A, prepared as in the Albayrak reference), (ii) PMM formed by polymerisation in the absence of Pluronic (batch B-control), and (iii) Pluronic alone, and the three spectra were then compared and analysed in detail.

In the case of the NMR experiment, Pluronic was added to the batch B-control sample just before the separation step, which was then immediately followed by the taking of the NMR spectrum. In this way, we compared the properties of a physical mixture of Pluronic with (already formed) PMM (from batch B-control), with that of the reaction mixture arising from a synthesis of PMM by polymerisation of the corresponding monomer in the presence of Pluronic (batch A). Since the Pluronic is not present during the polymerisation reaction stage in the batch B-control experiments, we postulate that here no PMM-Pluronic copolymer can possibly form, since there is no initiation or other activation of the polymers and no ongoing polymerisation reaction (and also the mixture is not

left for several hours to react, analysis following shortly afterwards).

The aim of this comparison between batch A and batch B-control was to investigate whether PMM synthesized in the presence of Pluronic (in batch A) gives any spectral data other than those obtained by a simple physical mixture of PMM obtained in the absence of Pluronic (in batch B-control) with later added Pluronic (as recorded in the NMR study), or with respect to a superposition of the spectra due to PMM and Pluronic (MALDI-TOF study). If the results indicate that, within reasonable limits of experimental error, the analyses of the two batches are equivalent, and also if no entirely new peaks are observed, I can conclude that in both cases only PMM and separate Pluronic polymers are present, and no copolymer between them.

The mass spectra of three samples (batch A, batch B-control and Pluronic® F68) are shown in Appendix A-1.

For Pluronic® F68 alone, the mass spectrum obtained is similar to that already reported in the literature (G. Gallet et al., Polymer 43 (2001) 1081-1094) with a bimodal distribution and 2 peaks of molecular weight 4800 and 11000.

For batch B-control, there is a series of peaks between 3800 and 9000 m/z with a mass difference between each of them of about 227 (table A). This can clearly be attributed to a MM 2.1.2-based polymer structure with a increasing number of monomer units.

For batch A, the mass spectrum appears as a clear superposition of the peaks due to Pluronic® F68 with those due to PMM from the batch B-control sample. This superposition is shown graphically in the overlay diagrams of Appendix A-2.

Furthermore, no new peaks (which might correspond to new copolymer species) are detected in the mass spectrum.

Also, no shift of existing peaks towards higher molecular weights is observed (this is another result which might be observed if PMM-Pluronic copolymers were being produced).

Although mass spectrometry is an essentially qualitative technique and cannot provide 100% certainty, it thus appears that when the polymerization of 1-ethoxycarbonyl, 1-ethoxycarbonylmethylene-oxycarbonylethane monomer is carried in the presence of Pluronic® F68 (batch A), the spectrum obtained by subjecting the batch-A sample to MALDI-TOF is equivalent to a superposition of the MALDI-TOF spectra obtained for separate samples of PMM (from batch B-control) and Pluronic® F68, i.e. it is strongly suggested that the conditions of Albayrak et al. simply give rise to a mixture of PMM and Pluronic® F68. There is no evidence that a covalent bond is formed to make a copolymer involving these two species.

The NMR method described in the "Materials and Methods" section of Appendix A does not allow a direct identification to be made of the polymer species present, because the NMR signal of protons in a molecule (to a first approximation) only depends on their immediate chemical environment.

The NMR signal obtained here quantifies the relative amounts of monomer units making up the different polymer types present, these being the oxyalkylene units (the only ones present in the polymer chain of pure Pluronic) and the methylidene malonate units (the only ones present in the polymer chain of pure PMM).

The NMR method used starts with a separation step whereby distilled water is added to precipitate some of the polymer species (after the freeze-dried batch is solubilized beforehand in tetrahydrofuran). By this means, it is expected that most of the hydrophobic polymers will precipitate and so form part of the pellet, and most of the hydrophilic polymers will remain in (essentially aqueous) solution. The logic of the experiment is that the formation of copolymers between hydrophobic PMM moieties and hydrophilic polyoxyalkylene moieties from Pluronic, if this occurs in the conditions of Albayrak et al (batch A in our study), would inevitably perturb the hydrophilic-hydrophobic balance.

For example, if the methylidene malonate polymerised in the presence of Pluronic (already present as a polymer at the beginning of the reaction), does indeed graft itself onto the Pluronic, the first copolymers to be formed would have a few methylidene malonate units attached. For reference, the average molecular mass of Pluronic® F68 is 8400. Small PMM chains grafted onto Pluronic would only add a relatively small amount to the overall mass of the Pluronic polymer.

If such PMM chains were grafted onto Pluronic, that would make a hypothetical PMM-Pluronic copolymer, which would still be rather hydrophilic, since it is mostly composed of hydrophilic Pluronic.

We would therefore expect that, if such copolymers formed, we would see an increase in the relative intensity of the NMR signals from the aqueous solution supernatant arising from the hydrophobic PMM monomer units, because the phenomenon of copolymerization with a hydrophilic polymer (Pluronic) would bring some of the hydrophobic PMM, normally

overwhelmingly in the precipitated pellet, into aqueous solution under the analytical conditions described.

However, we compared the results from batch A (polymerisation to form PMM in the presence of Pluronic, following Albayrak et al.), and from a physical mixture of Pluronic with the batch B-control sample (of pre-formed PMM polymer), prepared by mixing the batch B-control sample and Pluronic just before then separation step which is immediately followed by NMR analysis. In this comparison, it was observed that the relative intensities of signals due to oxyalkylene units and to methylidene malonate units in the supernatants and pellets in the two cases are almost the same, the differences in the observed relative intensities of signals not appearing to be significant.

It can therefore be concluded with a high degree of certainty that the polymerization to form PMM in the presence of Pluronic does not significantly modify the degree of hydrophilicity (or rather hydrophobicity) of PMM-containing polymers, as would be expected if copolymers were formed by combination with the hydrophilic Pluronic.

It has thus been observed that the NMR analysis of a pure physical mixture of PMM and Pluronic is equivalent (to a high degree of certainty) to that of PMM made by polymerization of the corresponding monomer in the presence of Pluronic, which suggests that in fact both samples, batch A and batch B, are the same, containing <u>separate and not covalently linked</u> PMM and Pluronic polymers.

The NMR analysis results <u>do not</u> support the hypothesis that PMM-Pluronic copolymers are formed as a result of the Albyrak et al method.

In conclusion, in both batches studied, no evidence of new species, which could be a copolymer, was found. To the contrary, the analytical results of the product of the Albyrak et al method are exactly equivalent to the results obtained by simply physically mixing pre-formed PMM polymer and Pluronic® F68 polymer just before the analysis. It is thus highly likely that the polymerization to form PMM in the presence of Pluronic® F68 simply gives rise to a physical mixture of discrete and not covalently bound PMM and Pluronic® F68 polymers.

Therefore, although we have looked for copolymer species arising from the conditions of Albayrak et al, no such copolymer species were observed directly, and there was also no indirect evidence pointing to the probable presence of such species.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: December 18th, 2003 By: Gérard RIESS

Appendix A

1) Materials and methods

Synthesis protocol:

Two batches of microparticles were synthesized, the first one in presence of Pluronic® F68 (batch A : Albayrak et al. example 15) and the second one without Pluronic® (Batch B - control), all the other parameters of the synthesis protocol being the same.

Batch A:

1 ml of monomer "1-ethoxycarbonyl, 1-ethoxycarbonylmethylene-oxycarbonylethane" (MM 2.1.2) was dispersed with a stirrer (Ika, Ultraturrax T25) in 100 ml of aqueous phosphate buffer (KH_2PO_4/Na_2HPO_4, 0,066 N, pH 5.5) which contained 3% Pluronic® F68 (Fluka, ref. 81 112) at 20°C for 60 minutes at 11000 rpm. Then, the reaction mixture was transferred into a flask that is equipped with a stirrer and polymerized for another 6 hours at room temperature while being stirred (300 rpm). Three batches were produced, mixed and freeze-dried.

Batch B (Control):

1 ml of monomer "1-ethoxycarbonyl, 1-ethoxycarbonylmethylene-oxycarbonylethane" (MM 2.1.2) was dispersed with a stirrer (Ika, Ultraturrax T25) in 100 ml of aqueous phosphate buffer (KH_2PO_4/Na_2HPO_4, 0,066 N, pH 5.5) at 20°C for 60 minutes at 11000 rpm. Then, the reaction mixture was transferred into a flask equipped with a stirrer and polymerized for another 6 hours at room temperature while while being stirred (300 rpm). Three batches were produced, mixed and freeze-dried.

Characterization Methods:

Mass spectrometry (MALDI-TOF):

The two freeze-dried batches (A and B-control) and Pluronic® F68 were solubilized in Tetrahydrofuran in the presence of HABA matrix (2-(4-hydroxyphenyl-azo)benzoic acid) (Fluka ref. 06788). Then, the samples were analyzed with a MALDI-TOF spectrometer (Matrix-Assisted Matrix Desorption/ Ionisation Time-of-Flight) OMNIFLEX Brucker. The mass-spectra obtained were compared.

NMR:

This NMR technique involves, as a first step, the separation of polymer species by precipitation using distilled water (after solubilization in tetrahydrofuran). PMM 2.1.2 is a hydrophobic polymer, insoluble in water (although some PMM 2.1.2 oligomers of low molecular weight do show some solubility). On the other hand, Pluronic® F68 is freely soluble in water up to a concentration of at least 100 g/L (see Chu B., Physical chemistry of polyalkylene block copolymer surfactants, V.M. Nace, M. Dekker, p78, 1996).

The freeze-dried batch A was resuspended in 30 ml tetrahydrofuran and precipitated in 300 ml distilled water. The freeze-dried batch B-control is resuspended in 30 ml tetrahydrofuran containing 9 grams of Pluronic® F68 and precipitated in 300 ml distilled water. Each suspension was centrifuged (Heraeus, Biofuge primo) at 8500 rpm (10000G) for 30 minutes. Then, the pellet (hydrophobic polymers) and the supernatant (hydrophilic polymers) were separated, freeze-dried, weighed and analyzed by proton NMR (Nuclear Magnetic Resonance Brucker AC-400MHz) after solubilization in deuterated chloroform ($CDCl_3$). The proportions of monomer residues making up the different polymer types present in the pellet and the supernatant were determined. Thus, the

relative intensities of NMR signals arising from oxyalkylene units (the only ones present in the polymer chain of pure Pluronic) and from methylidene malonate units (the only ones present in the polymer chain of pure PMM) were determined.

2) Results

Mass Spectra

Table 1, below, sets forth mass spectra comparison (MALDI-TOF) between batch A and batch B(control).

Table 1

Batch A				Batch B - control				Mass difference batch A /batch B
Mass Average 3 analysis		Standard deviation	Mass difference between 2 peaks	Mass Average 3 analysis		Standard deviation	Masss difference between 2 peaks	
4102,05	+/-	0,06%	_	4092,78	+/-	0,15%	_	0,23%
4317,40	+/-	0,05%	215,35	4307,57	+/-	0,18%	214,79	0,23%
4535,91	+/-	0,03%	218,51	4526,01	+/-	0,15%	218,45	0,22%
4758,83	+/-	0,00%	222,92	4753,65	+/-	0,00%	227,64	0,11%
4980,05	+/-	0,00%	221,22	4971,52	+/-	0,00%	217,87	0,17%
5205,74	+/-	0,01%	225,69	5194,78	+/-	0,07%	223,26	0,21%
5431,39	+/-	0,00%	225,65	5419,52	+/-	0,04%	224,75	0,22%
5657,56	+/-	0,01%	226,17	5645,43	+/-	0,03%	225,91	0,21%
5883,99	+/-	0,00%	226,43	5874,25	+/-	0,00%	228,82	0,17%
6111,12	+/-	0,00%	227,13	6099,27	+/-	0,03%	225,02	0,19%
6337,59	+/-	0,00%	226,47	6326,49	+/-	0,04%	227,22	0,18%
6565,05	+/-	0,01%	227,46	6552,99	+/-	0,03%	226,51	0,18%
6792,61	+/-	0,00%	227,56	6782,20	+/-	0,00%	229,21	0,15%
7019,87	+/-	0,01%	227,26	7008,42	+/-	0,03%	226,22	0,16%
7247,43	+/-	0,01%	227,56	7234,38	+/-	0,00%	225,96	0,18%
7474,69	+/-	0,01%	227,27	7462,69	+/-	0,02%	228,32	0,16%

NMR analysis

For the two batches, A and B(control), the weight and composition of each fraction (pellet and supernatant) are presented in Table 2 and Table 3, respectively.

Table 2: batch A

Pellet			Supernatant		
Weight* (g)	%methylidene malonate units**	%oxyalkylene units**	Weight* (g)	%methylidene malonate units**	%oxyalkylene units**
3.08	92.3	7.7	10.79	7.0	93.0

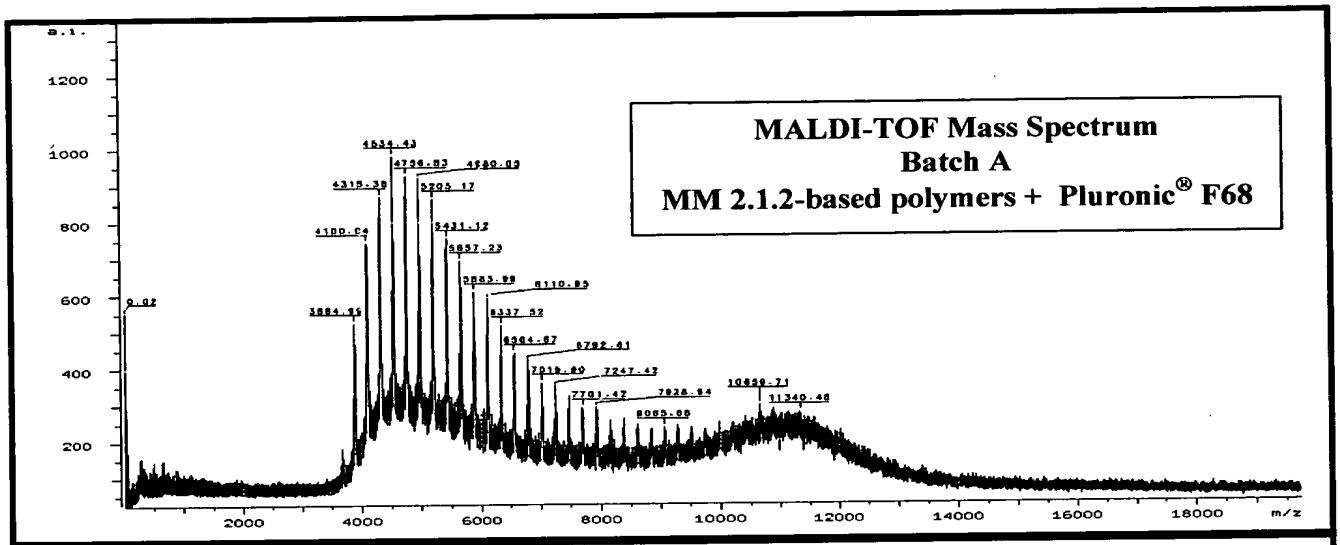
*: determined after freeze-drying
**: weight percentage determined by ^{1}H NMR

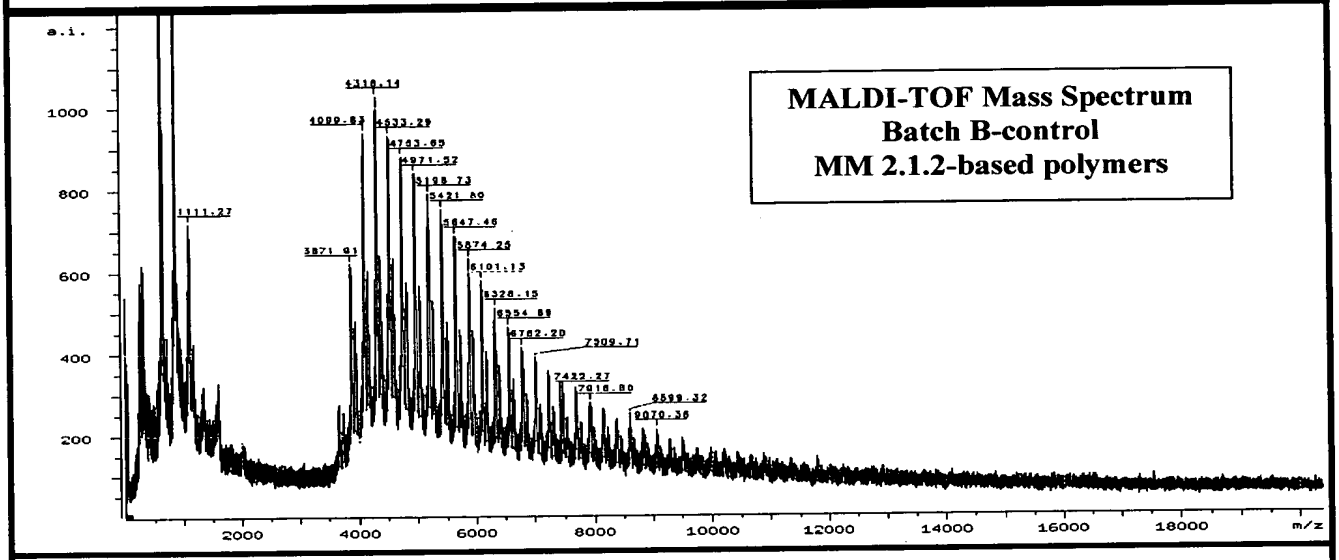
Table 3: batch B(control)

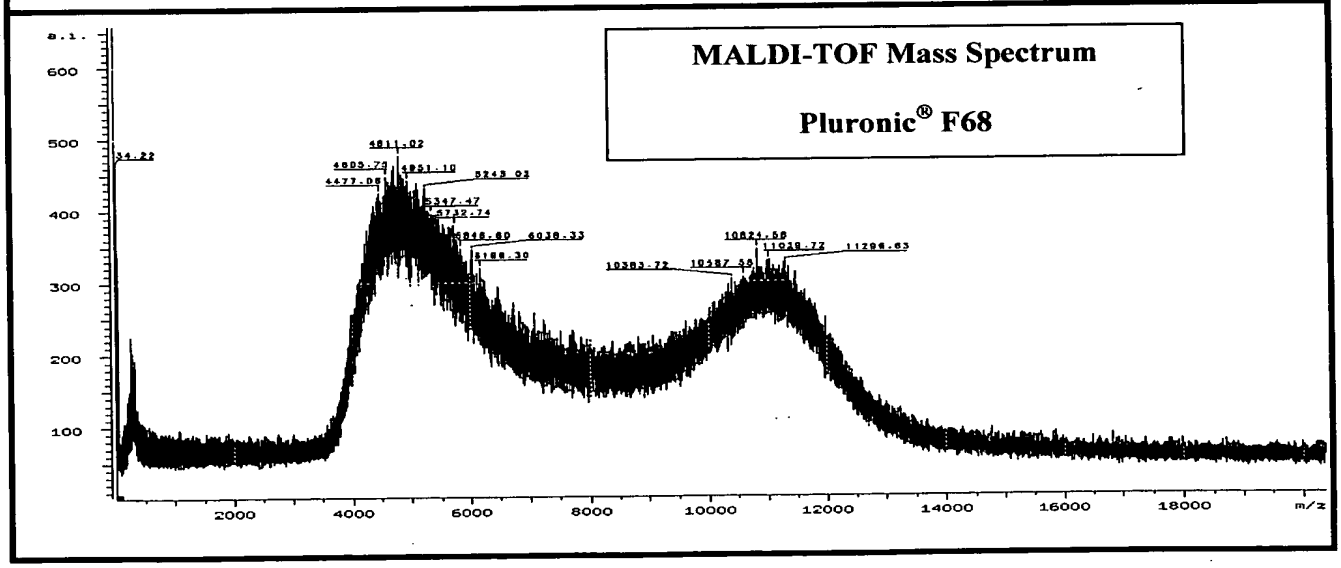
Pellet			Supernatant		
Weight* (g)	%methylidene malonate units**	%oxyalkylene units**	Weight* (g)	%methylidene malonate units**	%oxyalkylene units**
2.55	95.2	4.8	12.15	8.8	91.2

*: determined after freeze-drying
**: weight percentage determined by ^{1}H NMR

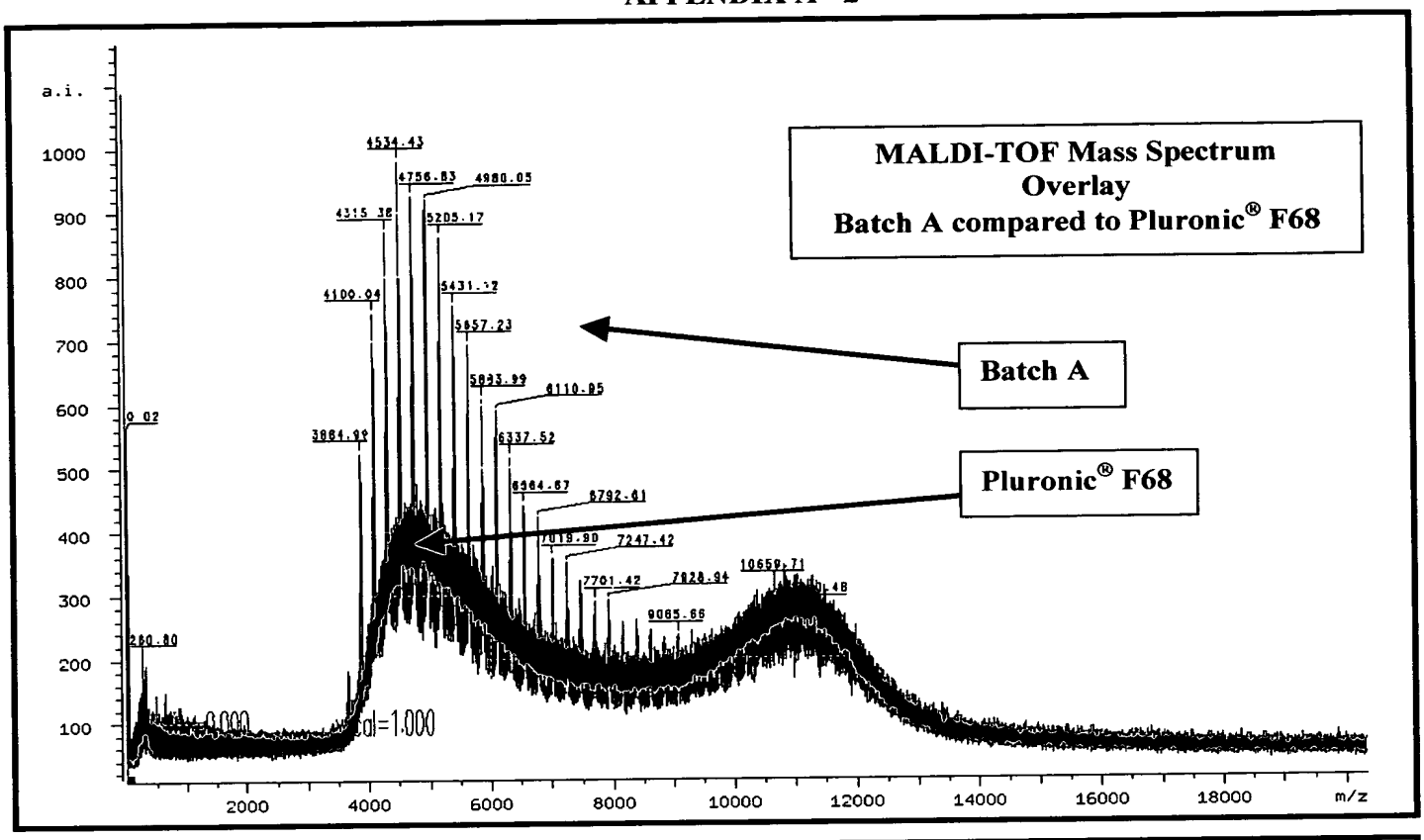
APPENDIX A -1

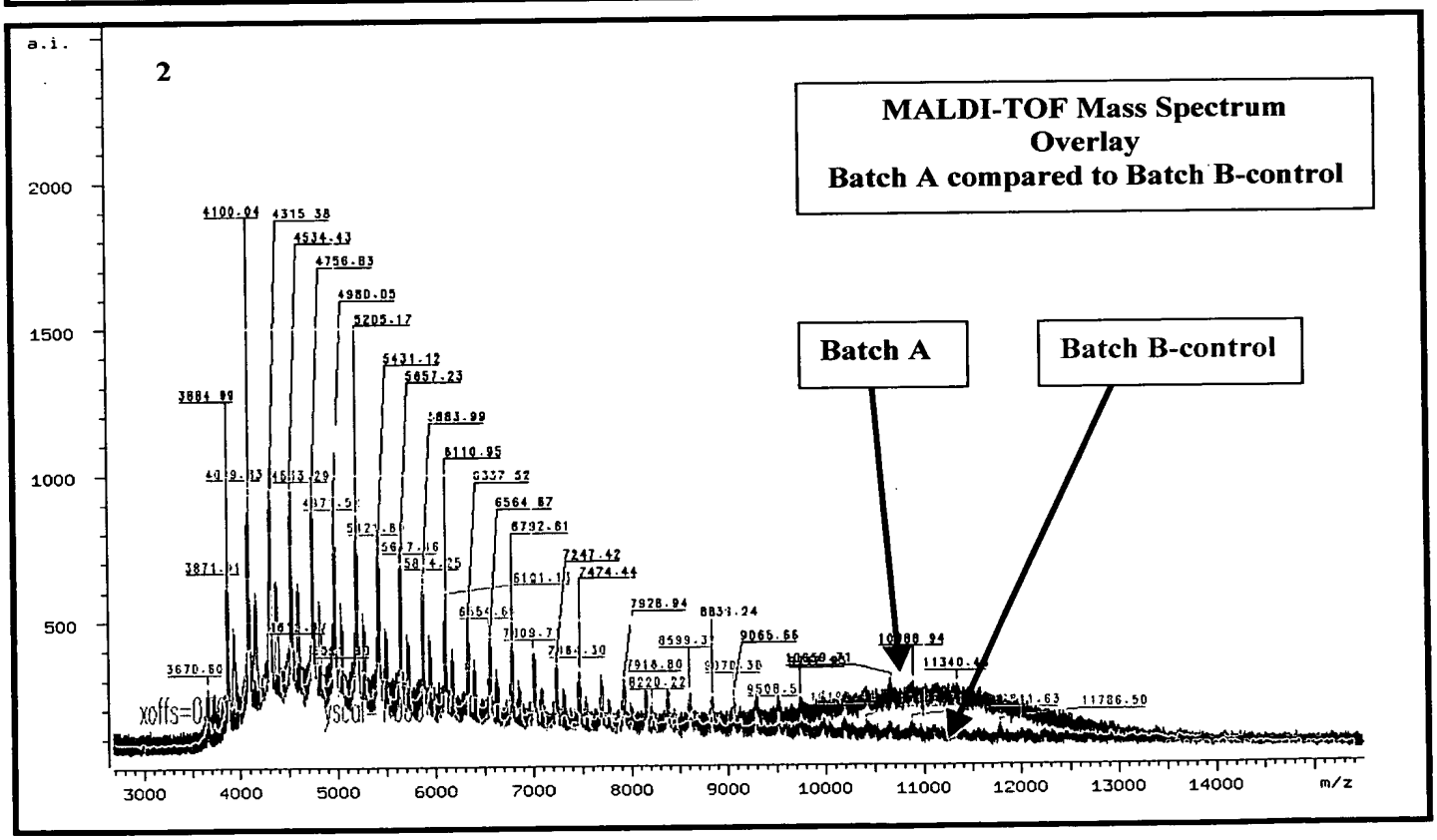

MALDI-TOF Mass Spectrum
Batch A
MM 2.1.2-based polymers + Pluronic® F68
a.i.
m/z


MALDI-TOF Mass Spectrum
Batch B-control
MM 2.1.2-based polymers
a.i.
m/z


MALDI-TOF Mass Spectrum
Pluronic® F68
a.i.
m/z

APPENDIX A - 2


a.i.
MALDI-TOF Mass Spectrum
Overlay
Batch A compared to Pluronic® F68
Batch A
Pluronic® F68
m/z


a.i.
2
MALDI-TOF Mass Spectrum
Overlay
Batch A compared to Batch B-control
Batch A
Batch B-control
m/z